10/678506

COFC

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313 on the date shown below:

August 24, 2006

Margaret H. Elfon , Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. UF-378C1
Patent No. 7,052,854 B2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Melker *et al.*
Issued	:	May 30, 2006
Patent No.	:	7,052,854 B2
For	:	Application of Nanotechnology and Sensor Technologies for Ex-vivo Diagnostics

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Cover Page:	See Communication Under 37 CFR 1.48(a) dated January 5, 2006:
"(75) Inventors: **Richard J. Melker,** Gainesville, FL (US); **Ronald L. Hayes,** Gainesville, FL (US); **Ka-Wang Kevin Wang,** Gainesville, FL (US); **Donn Michael Dennis,** Gainesville, FL (US)"	--(75) Inventors: **Richard J. Melker,** Gainesville, FL (US); **Ronald L. Hayes,** Gainesville, FL (US); **Ka-Wang Kevin Wang,** Gainesville, FL (US); **Donn Michael Dennis,** Gainesville, FL (US); **Charles R. Martin,** Gainesville, FL (US); **Jon D. Stewart,** Gainesville, FL (US)--
Cover Page, Abstract, Line 9:	Page 40, line 7:
"apparatus"	--"aptamers"--
Column 8, line 31:	Page 12, line 21:
"dichlroodiphenyltrichloroethane"	--dichlorodiphenyltrichloroethane--
Column 10, line 60:	Page 2 of Amendment filed August 26, 2005:
"Biophys. .2002"	--Biophys. J. 2002--
Column 10, line 64:	Page 2 of Amendment filed August 26, 2005:
"medium according"	--medium surrounding--
Column 11, line 24:	Page 3 of Amendment filed August 26, 2005:
"nanotubes based"	--nanotube based--
Column 13, line 33:	Page 3 of Amendment filed August 26, 2005:
"("or nanocap")"	--(or "nanocap")--
Column 13, line 48:	Page 4 of Amendment filed August 26, 2005:
"nonocaps"	--nanocaps--

Patent Reads:	**Application Reads:**
Column 14, line 54:	Page 20, line 13:
"Polymer Chemistry: An Invasion"	--Polymer Chemistry: An Invitation--
Column 15, line 4:	Page 20, line 25:
"polyorganosilioxane"	--polyorganosiloxane--
Column 15, line 11:	Page 20, lines 29:
"a salgenates"	--as algenates--
Column 15, line 23:	Page 21, line 8:
"in the templates"	--in the template--
Column 16, line 58:	Page 23, line 18:
"Discovery of"	--Diversity of--
Column 20, line 40:	Page 29, line 11:
"musculkoskeletal disorders"	--musculoskeletal disorders--
Column 21, line 13:	Page 30, line 7:
"the sample via to"	--the sample vial to--
Column 21, line 50:	Page 30, line 30:
"Bronchogenic carbinomas"	--Bronchogenic carcinomas--
Column 22, line 15:	Page 31, line 21:
"invention an include"	--invention can include--
Column 22, line 61:	Page 32, line 23:
"IN a rapid test"	--In a rapid test--

True and correct copies of pages 12, 20, 21, 23, 29-32, and 40 of the application as filed, pages 2-4 of the Amendment filed August 26, 2005, and Communication Under 37 CFR 1.48(a) filed January 5, 2006, which support the Applicants' assertion of the error on the part of the Patent Office accompany this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

Margaret H. Efron
Patent Attorney
Registration No. 47,545
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

MHE/wrc/an

Attachments: Certificate of Correction in duplicate;
Copies of pages 12, 20, 21, 23, 29-32, and 40 of the application as filed;
Copy of pages 2-4 of Amendment dated August 26, 2005; and
Copy of Communication Under 37 CFR 1.48(a) dated January 5, 2006.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

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ISSUE DATE : May 30, 2006
INVENTOR(S) : Richard J. Melker, Ronald L. Hayes, Ka-Wang Kevin Wang, Donn Michael Dennis, Charles R. Martin, Jon D. Stewart

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MAILING ADDRESS OF SENDER:
Saliwanchik, Lloyd & Saliwanchik
P.O. Box 142950
Gainesville, FL 32614-2950

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(source: intestines; diagnosis: lactose intolerance), isoprene (source: fatty acid; diagnosis: metabolic stress), methanethiol (source: methionine; diagnosis: intestinal bacterial overgrowth), methylethylketone (source: fatty acid; diagnosis: indoor air pollution/diet), O-toluidine (source: carcinoma metabolite; diagnosis: bronchogenic carcinoma), pentane sulfides and sulfides (source: lipid peroxidation; diagnosis: myocardial infarction), H_2S (source: metabolism; diagnosis: periodontal disease/ovulation), MeS (source: metabolism; diagnosis: cirrhosis), Me_2S (source: infection; diagnosis: trench mouth), αII-spectrin breakdown products and/or isoprostanes (source: cerebral spinal fluid, blood; diagnosis: traumatic or other brain injuries); prostate specific antigen (source: prostate cells; diagnosis: prostate cancer); and GLXA (source: gylcolipid in Chlamydia; diagnosis: Chlamydia).

Additional analytes/biomarkers that can be detected using the present invention include, but are not limited to, illicit, illegal, and/or controlled substances including drugs of abuse (*i.e.*, amphetamines, analgesics, barbiturates, club drugs, cocaine, crack cocaine, depressants, designer drugs, ecstasy, Gamma Hydroxy Butyrate – GHB, hallucinogens, heroin/morphine, inhalants, ketamine, lysergic acid diethylamide – LSD, marijuana, methamphetamines, opiates/narcotics, phencyclidine – PCP, prescription drugs, psychedelics, Rohypnol, steroids, and stimulants); allergens (*i.e.*, pollen, spores, dander, peanuts, eggs, and shellfish); toxins (*i.e.*, mercury, lead, other heavy metals, and *Clostridium Difficile* toxin); carcinogens (*i.e.*, acetaldehyde, beryllium compounds, chromium, dichlorodiphenyltrichloroethane (DDT), estrogens, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and radon); and infectious agents (*i.e.*, *Bordettella bronchiseptica*, citrobacter, *Escherichia coli*, hepatitis viruses, herpes, immunodeficiency viruses, influenza virus, *Listeria*, micrococcus, mycobacterium, rabies virus, rhinovirus, rubella virus, *Salmonella*, and yellow fever virus).

The term "bodily fluid," as used herein, refers to a mixture of molecules obtained from a patient. Bodily fluids include, but are not limited to, exhaled breath, whole blood, blood plasma, urine, semen, saliva, lymph fluid, meningal fluid, amniotic fluid, glandular fluid, sputum, feces, sweat, mucous, and cerebrospinal fluid. Bodily fluid also includes

The outside diameter of the nanotube can be controlled by varying the pore diameter of the template membrane, the length of the nanotube can be controlled by varying the thickness of the template membranes, and the inside diameter of the nanotube can be controlled by varying the immersion time in the sol.

Polymer nanotubes can be prepared from many substances that are composed of monomer units. "Monomer units," as used herein, refers to the individual moieties that are repeated to form "polymers." Multiple monomer units are covalently attached when tin the form of a backbone of a polymer. Polymers that are made from at least two different types of monomer units are referred to as "copolymers." Polymerizing or copolymerizing describes the process by which multiple monomers are reacted to form covalently linked monomer units that form polymers or copolymers, respectively. A discussion of polymers, monomer units, and the monomers from which they are made may be found in Stevens, Polymer Chemistry: An Invitation, 3rd ed., Oxford University Press (1999).

Polymeric nanotubes can be prepared using a solution deposition method as described in Depak, V.M. and C.R. Martin, "Preparation of Polymeric Micro- and Nanostructures Using a Template-Based Deposition Method," *Chem. Mater.*, 11:1363-1367 (1999). This method entails depositing a solution of the desired polymer within the pores of the template membrane and allowing the solvent to evaporate. In addition, polymer nanotubes can be prepared by polymerizing a monomer of a monomer within the pore as described by Martin, C.R., "Template Synthesis of Electronically Conductive Polymer Nanostructures," *Acc. Chem. Res.*, 28:61-68 (1995).

Preferred polymers include polystyrene, polyorganosiloxane, poly(methyl methacrylate), polystyrene, polylactic acids, and other biodegradable polymers, acrylic latexes, polyorganosiloxane, cellulose, polyethylene, poly(vinyl chloride), poly(ethyl methacrylate), poly(tetrafluoroethylene), poly(4-iodostyrene/divinylbenzene), poly(4-vinylpyridine/divinylbenzene), poly(styrene/divinyl benzene), crosslinked melamine particles, phenolic polymer colloids, polyamide 6/6, natural rubber, naturally occurring biopolymers such as algenates and collagen, or mixtures thereof.

Functionalization of Nanoparticle Surface

Methods used to functionalize a nanoparticle surface, in accordance with the present invention, depend on the composition of the nanoparticle and are well known in the art. For example, functionalization of silica nanoparticles is accomplished using silane chemistry. The detecting means and/or the surrogate marker can be attached to the surfaces of the nanoparticle by attaching them to the surface of the nanoparticle while the nanoparticle is still embedded within a template. Alternatively, while the nanoparticle is embedded in the template, a hydrolytically unstable silane is reacted with surface silanol sites on the nanoparticle to obtain covalent oxygen/silicon bonds between the surface and the silane. Either the detecting means and/or the surrogate marker can then be attached to the surface of the nanoparticle after dissolution of the template.

The surface of polymer-based nanoparticles can also be functionalized using well-known chemical methods. For example, the methods employed for polylactide synthesis allow for differential end-functionalization. Polymerization occurs by an insertion mechanism mediated by Lewis acids such as Sn^{2+} whose bonds with oxygen have significant covalent character. An alcohol complexed with the metal ion initiates polymerization, which continues by stepwise ring-opening of the lactide monomers to generate a new alkoxide-metal complex capable of chain growth. The polymer molecular weight can be controlled by the molar ratio of initiating alcohol to the lactide monomer. The resulting polyester possesses directionality with a hydroxyl terminus (from the first monomer) and either a detecting means and/or surrogate marker at the ester terminus determined by the structure of the initiating alcohol. The latter can contain a variety of detecting means and/or surrogate markers.

Additionally, the detecting means and/or surrogate marker can be introduced by copolymerization. Natural amino acids re sterically similar to lactic acid but offer a variety of functional groups on their side-chains (-OH, $-CO_2H$, $-NH_2$, -SH, *etc.*). Monomer derived from an amino acid and lactic acid can be synthesized by standard methods and used for random copolymerization with lactide.

By functionalizing the nanoparticle with either a detecting means and/or surrogate marker, the present invention provides nanostructure-based assemblies that can

fragment through established immunochemical methods; and fusion proteins, generated from hybrid genes developed and expressed through recombinant methods.

According to the present invention, other contemplated means for detecting a target analyte/biomarker include antibodies, antigens, haptens and nucleic acid probes with site-directed effectors (*i.e.*, fluorophores). DNA, including branched DNA, can also be used in accordance with the present invention as a means for detecting target analytes/biomarkers. For example, it has been shown that particular proteins recognize and bind to specific sites on the DNA. See Seeman, *Clin. Chem.*, 39:722 (1993).

The present invention preferably utilizes aptamers to non-invasively detect drugs, biomarkers, and other analytes in exhaled breath and other bodily fluids, such as blood. In a preferred embodiment, the invention includes aptamers in combination with nanotechnology (*i.e.*, nanotubes) to provide an effective method for signaling the presence of a target analyte/biomarker in bodily fluids, particularly in blood.

The discovery of the SELEX™ (Systematic Evolution of Ligands by EXponential enrichment) methodology enabled the identification of aptamers that recognize molecules other than nucleic acids with high affinity and specificity (Ellington and Szostak, "In vitro selection of RNA molecules that bind specific ligands," *Nature*, 346:818-822 (1990); Gold *et al.*, "Diversity of oligonucleotide functions," *Ann. Rev. Biochem.*, 64:763-797 (1995); Tuerk and Gold, "Systematic evolution of ligands by exponential enrichment - RNA ligands to bacteriophage-T4 DNA-polymerase," *Science*, 249:505-510 (1990)). Aptamers have been selected to recognize a broad range of targets, including small organic molecules as well as large proteins (Gold *et al.*, *supra.*; Osborne and Ellington, "Nucleic acid selection and the challenge of combinatorial chemistry," *Chem. Rev.*, 97:349-370 (1997)).

The aptamers derived from the SELEX methodology may be utilized in the present invention. The SELEX methodology enables the production of aptamers, each of which have a unique sequence and the property of binding specifically to a desired target compound or molecule. The SELEX methodology is based on the insight that nucleic acids have sufficient capacity for forming a variety of two- and three-dimensional structures and sufficient chemical versatility available within their monomers to act as

strep throat), endocrine disorders (*i.e.*, congenital adrenal hyperplasia, diabetes, hypoglycemia, hyperparathyroidism, hypoparathyroidism, and Cushing's syndrome), eye disorders (*i.e.*, retinoblastoma, uveitis, Lebers optic neuropathy, keratoconus), genetic disorders (*i.e.*, Marfan's syndrome, porphyries, Huntington's disease, normal pressure hydrocephalus (NPH), Wilson's disease), gynecologic disorders (*i.e.*, polycystic ovarian syndrome, endometriosis), immune disorders (*i.e.*, AIDS, Addison's disease, Lupus, Sjogren's syndrome), infectious diseases (*i.e.*, bacterial (rickettsial diseases, anthrax, endocarditis, salmonellosis), viral (chickenpox, herpes, influenza, pneumonia, shingles, West Nile virus), fungal (aspergillosis), parasitic (malaria, scabies, pinworms), prion (Creutzfeldt Jakob Disease)), metabolism disorders (fatty oxidation disorders, glycogen storage disorders I and II, glutaric acidemia), musculoskeletal disorders (osteoporosis), neurological disorders (Alzheimer's disease, meningitis, demyelinating diseases), respiratory conditions, and urological disorders (hemolytic uremic syndrome, urinary tract infections).

Example 1—Diagnosis of Traumatic Brain Injury (TBI)

The present invention provides methods for diagnosing acute and/or chronic neurological diseases and disorders (*i.e.*, Alzheimer's disease, Parkinson's disease) and other clinical conditions by detecting *in vitro* analytes/biomarkers of oxidative stress. For example, it is known that certain αII-spectrin breakdown products, including isoprostane, levels increase in cerebral spinal fluid and blood after traumatic brain injury.

In accordance with the present invention, nanostructure-based assemblies are created in which the detecting means is designed to specifically detect and localize the assembly to isoprostanes and/or αII-spectrin breakdown products. In a preferred embodiment, the detecting means is an aptamer designed to bind to isoprostanes and/or αII-spectrin breakdown products. A sample of a patient's bodily fluid (*i.e.*, blood or cerebral spinal fluid) is placed into a sealed vial containing the nanostructure-based assemblies designed as described above.

In one embodiment, the sample is incubated at an elevated temperature to allow any surrogate markers that were released from the nanostructure-based assemblies to

diffuse out of the liquid phase into the "headspace" (gas phase) within the sealed vial. Under constant conditions of temperature, pressure, and equilibration time, the vapor phase in the sample vial is sampled and separated on a suitable gas chromatographic column. The surrogate markers are detected using flame ionization detector or nitrogen phosphorous detector.

In another embodiment, an "electronic nose" is used to detect and measure the amount of surrogate marker released in the sample vial to assess whether the patient suffers from traumatic brain injury. As contemplated by the subject invention, the electronic nose can include the following components: (a) a sensor having an array of polymers capable of detecting the presence of the surrogate marker in the headspace of the vial, wherein the sensor responds to the surrogate marker by changing the resistance in each polymer resulting in a pattern change in the sensor array; (b) a processor for receiving the change in resistance, comparing the change in resistance with a previously measured change in resistance, and identifying the presence of the surrogate marker from the pattern change, and (if requested) the concentration of the surrogate marker from the amplitude. In a related embodiment, the sensor can include measuring circuitry and an output device can be included (*i.e.*, screen display, audible output, printer). The processor can include a neural network for comparing the change in resistance with a previously measured change in resistance to find a best match.

By measuring isoprostane levels and/or αII-spectrin breakdown products using the nanostructure-based assemblies of the invention, a clinician can not only identify if a patient is suffering from TBI, but once diagnosed, a clinician can follow the course of the brain injury. Moreover, by continuously testing samples of bodily fluid in accordance with the present invention, it is possible to evaluate the efficacy of interventions in real-time for treating TBI. Accordingly, the method of the present invention can also evaluate pharmacodynamics and pharmacokinetics for drug interventions in individuals.

Example 2—Diagnosis of Bronchogenic Carcinoma

In an embodiment, a nanostructure-based assembly of the present invention can be designed to detect bronchogenic carcinoma. Bronchogenic carcinomas produce

carcinoma metabolites that cause the occurrence of O-toluidine in exhaled breath. The detecting means of the nanostructure-based assembly can be in the form of an aptamer. Using routine techniques, the aptamer can be designed so that it is specific for O-toluidine (O-toluidine-aptamer). The O-toluidine-aptamer can be linked to a nanoparticle using functionalization methods as described above. The nanoparticle contains a surrogate marker that would be released in the presence of O-toluidine. Upon exposing the nanostructure-based assembly to a sample of bodily fluid (*i.e.*, exhaled breath) suspected of containing O-toluidine, the O-toluidine-aptamer specifically binds to any O-toluidine present in the sample and causes the release of the surrogate marker to generate a signal that O-toluidine is present in the bodily fluid sample.

In one embodiment, sensor technology used to detect the surrogate marker in the sample has the following components: (a) a surface-acoustic wave sensor capable of detecting the presence of the surrogate marker in the mixture of bodily fluid (*i.e.*, expired breath) and the nanostructure-based assembly, wherein the sensor responds to the surrogate marker by a shift in the resonant frequency; (b) an oscillator circuit having the sensor as an active feedback element; (c) a frequency counter in communication with the oscillator circuit to measure oscillation frequency which corresponds to resonant frequency of the sensor; and (d) a processor for comparing the oscillation frequency with a previously measured oscillation frequency with a previously measured oscillation frequency of the surrogate marker and determining presence and concentration of the surrogate marker therefrom. The sensor technology of the present invention can include measuring circuitry and an output device (*i.e.*, screen display, audible output, and printer).

The processor can include a neural network (not shown) for pattern recognition. Artificial Neural Networks (ANNs) are well understood by the skilled artisan. ANNs are self-learning; the more data presented, the more discriminating the instrument becomes. By running many standard samples of bodily fluids and storing the results in computer memory, the application of ANN enables the sensor technology to "understand" the significance of the sensor array outputs better and to use this information for future analysis. "Learning" is achieved by varying the emphasis, or weight, that is placed on the

output of one sensor versus another. The learning process is based on the mathematical, or "Euclidean," distance between data sets. Large Euclidean distances represent significant differences in sample-to-sample surrogate marker characteristics.

Thus, a time- and cost-efficient test for the presence of bronchogenic carcinoma is provided.

Example 3—Diagnosis of Prostate Cancer

In another embodiment, a detecting means is designed for a biomarker of a specific cancer, *i.e.*, prostate cancer. Prostate cancers produce a protein, prostate specific antigen (PSA). In a preferred embodiment, the detecting means is an aptamer designed to specifically bind to PSA (PSA-aptamer). The PSA-aptamer can be attached to a nanoparticle using functionalization methods as described above. The nanoparticle also includes a surrogate marker that is released in the presence of PSA. In one embodiment, the PSA-aptamer and the surrogate marker are attached to the surface of the nanoparticles using functionalization methods as described above. The nanostructure-based assembly is introduced to a sample of bodily fluid (*i.e.*, blood) to identify the presence of PSA. Where PSA is present in the bodily fluid sample, the PSA-aptamer will bind to PSA and affect the release of the surrogate marker form the nanoparticle to signal the presence and concentration of PSA in the bodily fluid sample.

In a preferred embodiment, the nanostructure-based assembly is composed of a hollow nanoparticle. The detecting means, *i.e.*, PSA-apatamer, is attached to an end-cap that fits onto an opening of a nanoparticle. The nanoparticle preferably encapsulates a surrogate marker. In a rapid test for the presence of prostate cancer, or a recurrence of prostate cancer, the PSA-nanostructure-based assembly is mixed with a sample of bodily fluid (*i.e.*, exhaled breath, exhaled condensates with proteins). The surrogate marker is released from the nanoparticle after PSA (the biomarker of interest) interacts with the PSA-aptamer and "uncaps" the nanoparticle. Using any of a number of previously disclosed sensor technologies, the surrogate marker is detected in the sample of exhaled breath to indicate the presence and/or concentration of PSA in the sample.

Abstract of the Disclosure

Systems and methods for the *ex vivo* diagnostic analysis of samples of bodily fluids, including exhaled breath and blood. The present invention uses nanostructure-based assemblies in combination with sensor technology to provide an efficient and accurate means for identifying the presence of a target analyte/biomarker in a sample of bodily fluid. In a preferred embodiment, the nanostructure-based assemblies of the present invention include detecting means such as RNA oligonucleotide chains or "aptamers" and releasable surrogate markers such as DMSO.

In the specification

Please insert the following paragraphs at page 16, line 5:

Depending upon the application, various types of sensors, for example, aptamers, antibodies/proteins, peptides, or high affinity ligands, can be linked to the uncapping/discharge mechanism of the nanocap-nanostructure-based assemblies of the invention. Thus, the uncapping mechanism can be linked to detection by the sensors on the nanocap-nanotube structure of surface markers on cells types (e.g., cancer cells), proteins in the blood (e.g., PSA for prostate cancer) or drugs in the body (e.g., illicit drugs or therapeutic drugs). These may or may not require the use of energy-bearing biomolecular motors such as, but not limited to, the actin-based system (Dickinson R. B. and Purich D. L., Biophys. J. 2002 82:605-617).

In another embodiment, the nanocap (or "end-cap") is attached by electrostatic attraction between the nanocap and the nanotube. The cap is released in response to a change in the ionic strength of the medium surrounding the nanotube. Alternatively, the cap can be held on by hydrogen bonding or by acid and/or basic sites on the nanocap/nanotube. The cap is released by a change in the pH or the surrounding medium. The cap may also be held on by covalent bonds that can be cleaved by a specific enzyme, for example, a hydrolase enzyme.

The sensors can be designed to initiate release of payload contents (such as a surrogate marker) upon detecting stimuli. Such stimuli can include physical stimuli, for example, the temperature, pressure, velocity or acceleration of the nanoparticle; biological stimuli, for example, the presence of normal or abnormal cell types, cellular surface antigens, proteins, oligonucleotides, or toxins; or chemical stimuli, for example, pH, ionic strength, hydration state, redox state, or the presence of therapeutic agents, or toxic drugs such as nerve agents.

For example, one can achieve safe and effective intracellular surrogate marker release by attaching the nanocap to the nanotube with covalent bonds (e.g., S--H bonds) that are broken when a specific chemical signal (e.g., high reducing atmosphere of the cytoplasmic environment of the interior of a mammalian cell) is encountered. The ability to incorporate different types of sensor mechanisms for removal of the cap is an extremely powerful approach to the delivery and

release (or uptake) of payload contents in an event- and site-specific manner. Specifically, by linking the uncapping mechanism to various sensing modes, the nanotube based surrogate marker transport systems can be used to diagnose, treat, and monitor health status. For example, smart nanotubes can detect the appearance of cancer antigens on the walls of cancer cells, cause uncapping which in turn releases an indicator, which in turn makes the urine a distinct color or releases a nontoxic marker which can be readily detected in the breath, and thereby notifies the patient or his/her physician that a cancer cell(s) was encountered in his/her body.

Nanotube technology provides a method for delivering surrogate markers. In one embodiment, this is achieved using nanocaps that are firmly bound to the nanotube when the assembly is outside of the cell but are released, thus opening the nanotube and making the surrogate marker available, when the assembly is partitioned into the cell. For example, this can be accomplished using disulfide chemistry to couple the nanoparticle cap to the nanotube. The disulfide link between the nanotube and its nanocap is ideal because all living cells maintain a reducing environment within their cytoplasm. This contrasts with the oxidizing environment found outside the cell. The tripeptide glutathione (-gluatamyl-cysteinyl-glycine) plays a key role in this process. In its reduced form, glutathione possesses a free sulfhydryl capable of reducing disulfide bonds, forming a disulfide-linked glutathione dimer in the process. This species, in turn, is reduced by nicotinamide-dependent enzymes.

Please insert the following paragraphs at page 18, line 26:

The end-cap (or "nanocap") can be used to impart several novel functions and degrees of intelligence to the nanotube-nanocap delivery system. These include the sealing of the payload contents (such as the surrogate marker) within the nanotube in a cost-effective manner.

The nanocap can also provide a mechanism whereby the nanotube payload contents can be selectively released. For example, when used for the in-vivo delivery of a surrogate marker, the nanotube can be designed to release its payload either at the surface of the target cell or within its cytoplasm. This may be achieved by sensing a chemical, physical or biological signal

present in the local environment. Alternatively; a remote external energy source, such as ultrasonic irradiation, can be used to selectively release the payload from the nanotube. Time-controlled degradation of the biomaterials used to construct the nanotube and/or nanocaps can also provide a release mechanism.

FACSIMILE COVER SHEET

SALIWANCHIK, LLOYD & SALIWANCHIK
A Professional Association
P.O. Box 142950
Gainesville, FL 32614-2950

Telephone (352) 375-8100
Facsimile (352) 372-5800

TO: Examiner Jacqueline A. DiRamio

FROM: Margaret Efron

COMPANY: U.S. Patent Office
Art Unit 1641

DATE: January 5, 2006

FAX NO.: (571) 273-8300

NO. OF PAGES (INCLUDING COVER SHEET): 45

SUBJECT/MESSAGE:

Re: Serial No. 10/678,506; filed October 2, 2003
Docket No. UF-378C1

1) Communication under 37 CFR 1.48(a) (2 pages);
2) Petition & Request for Correction of Inventorship Under 37 CFR 1.48(a) (3 pages)
3) Statement and Certificate Under 37 CFR §3.73(b) (2 pages);
4) Two Assignments (13 pages);
5) Statements from inventors being added (2);
6) Statements from existing inventors (4); and
7) Newly executed Declaration and Power of Attorney forms (18 pages)

THIS IS AN OFFICIAL DOCUMENT!
PLEASE DELIVER TO EXAMINER DiRAMIO IMMEDIATELY

If you do not receive all pages or if any transmission is not legible, call the sender at (352) 375-8100.

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I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on the date shown below:

January 5, 2006

Margaret Efron, Patent Attorney

COMMUNICATION
Serial No. 10/678,506
Docket No. UF-378C1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Jacqueline DiRamio
Art Unit	:	1641
Applicant(s)	:	Richard J. Melker, Ronald L. Hayes, Ka-Wang Kevin Wang, Donn Michael Dennis
Serial No.	:	10/678,506
Filing Date	:	October 2, 2003
Conf. No.	:	1941
For	:	Novel Application of Nanotechnology and Sensor Technologies for Ex-vivo Diagnostics

Mail Stop Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

COMMUNICATION UNDER 37 CFR 1.48(a)

Sir:

This Communication and attached documents are being submitted to the U.S. Patent to request correction of inventorship in the above-mentioned application.

Attached herewith are the following documents:

1) Petition and Request for Correction of Inventorship Under 37 CFR §1.48(a);
2) Certificate Under 37 CFR §3.73(b) of Assignee;
3) Statement submitted by added inventors (Martin and Stewart) (2);
4) Statement submitted by existing inventors (Melker, Hayes, Wang, Dennis) (4);

5) Newly executed Declaration (37 CFR §1.63) and Power of Attorney form (6); and
6) Courtesy copy of recorded assignment for the Examiner's convenience.

Respectfully submitted,

Margaret Efron
Patent Attorney
Registration No. 47,545
Phone No.: (352) 375-8100
Fax No. : (352) 372-5800
Address : P.O. Box 142950
Gainesville, FL 32614-2950

MHE/la

Attachments: as stated above

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on the date shown below:

January 5, 2006

Margaret Efron, Patent Attorney

PETITION TO ADD INVENTOR
UNDER 37 C.F.R. 1.48
Docket No. UF-378C1
Serial No. 10/678,506

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Jacqueline DiRamio
Art Unit	:	1641
Applicant(s)	:	Richard J. Melker, Ronald L. Hayes, Ka-Wang Kevin Wang, Donn Michael Dennis
Serial No.	:	10/678,506
Filing Date	:	October 2, 2003
Conf. No.	:	1941
For	:	Novel Application of Nanotechnology and Sensor Technologies for Ex-vivo Diagnostics

Mail Stop: Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PETITION & REQUEST FOR CORRECTION OF INVENTORSHIP UNDER 37 CFR 1.48(a)

Sir:

It is respectfully requested that the inventorship of the above-identified application be corrected. Authority for this petition and the correction of inventorship is found in 37 C.F.R. 1.48(a), reproduced below.

> 37 C.F.R. § 1.48 Correction of inventorship in a patent application, other than a reissue application

(a) If the inventive entity is set forth in error in an executed Section 1.63 oath or declaration in an application, other than a reissue application, and such error arose without any deceptive intention on the part of the person named as an inventor in error or on the part of the person who through error was not named as an inventor, the application may be amended to name only the actual inventor or inventors. When the application is involved in an interference, the amendment must comply with the requirements of this section and must be accompanied by a motion under Section 1.634. Such amendment must be accompanied by:

(1) A request to correct the inventorship that sets forth the desired inventorship change;

(2) A statement from each person being added as an inventor and from each person being deleted as an inventor that the error in inventorship occurred without deceptive intention on his or her part;

(3) An oath or declaration by the actual inventor or inventors as required by Section 1.63 or as permitted by Section 1.42, 1.43 or 1.47;

(4) The processing fee set forth in Section 1.17(i); and

(5) If an assignment has been executed by any of the original named inventors, the written consent of the assignee (see Section 3.73(b)).

Charles Martin and Jon D. Stewart were unintentionally, and without deceptive intent, not originally included on the application as coinventors. The applicants respectfully request the correction of inventorship.

Accompanying this Petition and Request for Correction of Inventorship are the following:

(1) A petition including a statements from the individuals being added as co-inventors as well as statements from existing inventors that the error in inventorship occurred without deceptive intention on their part ;
(2) A declaration under Section 1.63 by the actual inventors;
(3) The fee set forth in Section 1.17(i); and
(4) The written consent of the assignee (see Section 3.73(b)).

Please charge $130.00 to Deposit Account 19-0065. Two copies of this sheet are enclosed. The Commissioner is also authorized to charge any additional fees that may be required by this paper to Deposit Account No. 19-0065.

Respectfully submitted,

Margaret Efron
Patent Attorney
Registration No. 47,545
Phone No.: (352) 375-8100
Fax No. : (352) 372-5800
Address : P.O. Box 142950
Gainesville, FL 32614-2950

MHE/la

Attachments: Written Consent Certificate Under 37 CFR §3.73(b) of Assignee
Executed Statements of all co-inventors (6); and
Executed Declaration and Power of Attorney forms of all inventors (6)